Effects of perfluorononanoic acid (PFNA) on the metabolic profiling of rat serum by UHPLC-ESI-Q-TOF MSMS

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Human relevant dose of endocrine disrupting chemicals effect on the rat plasma metabolome

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Background

Endocrine disrupting chemicals (EDC) are chemicals disturbing the hormones of the body. Many chemical compounds are under suspicion of being an endocrine disruptor. The effect of a variety of EDC has been tested for changes in male and female hormone composition.
In order to understand the effect of EDC on the metabolome an analytical platform has been established. The method focuses separating the compounds from the plasma into three groups: phospholipids, lipids and a fraction containing hormones, organic acids etc. thereby avoiding ion supression.
The main goal of the present study is to identify if a human relevant dose of EDC will have an effect on the rat metabolome. A human relevant dose of a possible EDC, perflournonanoic acid, was given to a group of rats. To another group PFNA and 12 other EDCs were given. These two groups were compared to a group given only the 12 EDC's and a control group.
By separating the metabolites into three fractions and using high resolution mass spectrometry it is possible to achieve high level of information of how EDC affects the rat metabolome.

Cocktail of EDC

Name	Ratio	mg/L	mg/400 mL
Bisphenol A	0,005	5	2
Butyl paraben	0,257	257	103
DBP d=1.05	0,030	30	12
DDE	0,003	3	1
DEHP d=0.98	0,043	43	17
Epoxiconazole	0,025	25	10
Linuron	0,002	2	1
MBC	0,194	194	78
OMC d=1.01	0,340	340	136
Prochloraz	0,031	31	12
Procymidone	0,044	44	18
Vinclozolin	0,026	26	11


Glabridin


Bergamottin

Plasma analysis

- 100 µl plasma is extracted with 300 µl icecold acetonitrile and left in the freezer for 20 min
- The sample is centrifuged at 10000 g and supernatant removed
- A SPE hybrid column (Supleco, Sigma-Aldrich, USA) is activated at the supernatant added
- The throughput (T) is collected and the phospholipid eluate with 300 µl 10 % NH_4OH in methanol and collected in another fraction
- The T is evaporated by a gentle stream of nitrogen and the dried compound extracted with three different solvents
- Firstly, in 200 µl heptane
- Secondly, in 200 µl methanol
- Lastly, in 200 µl 5 % acetonitrile
- The heptane is evaporated using a gentle stream of nitrogen and the dried compound ressolved in 200 µl 50:50 acetonitrile:isopropanol
- The method separates the plasma sample into four different fractions, analyzed by two different LC-MS methods - a hydrophilic and hydrophobic LC system.

Analytical setup

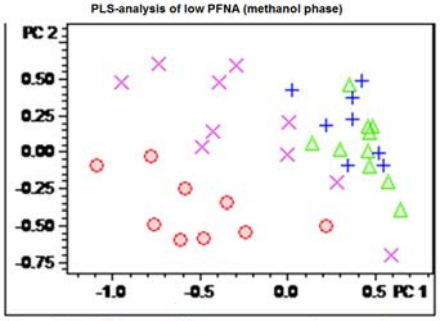
Dionex 3000 series UHPLC system combined with a Bruker Daltonics maxis qTOF instrument.

A: Water with 5 mM NH_4OH and 0.1 % formic acid
B: Acetonitrile with 0.1 % formic acid
Hydrophilic gradient system
0 min, 0 % B – 1 min, 0 % B – 3 min, 5 % B – 10 min, 100 % B – 12, 100 % B – 12.1, 0 % B – 14, 0 % B
Hydrophobic gradient system
0 min, 70 % B – 1 min, 70 % B – 3 min, 75 % B – 8 min, 100 % B – 10, 100 % B – 10.1, 70 % B – 12, 70 % B

The column used was a poreshell EC-C8 column from supleco (Agienlent Technologies, MO, USA)

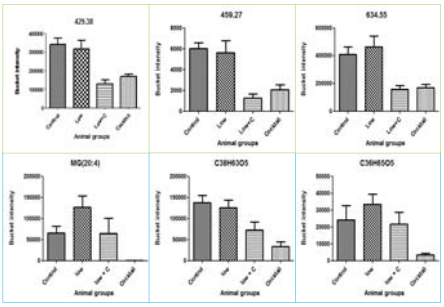
Perflourononanoic acid is a compound primarily used in packing materials. The compound is a surfactant used to lower the water tension within packing material. PFNA have been shown to have an endocrine disrupting effect cellular assays and on zebra fish.

PLS-analysis of low PFNA (methanol phase)



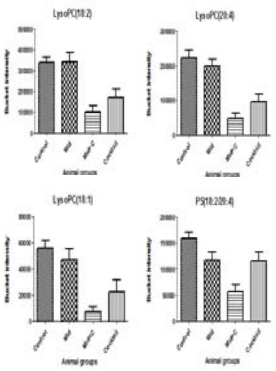
PLS-DA of low PFNA concentration. As shown on the PLS plot is there similarities between the low PFNA with cocktail and the cocktail. Furthermore, is there difference between these two groups and the control group.

Human relavant dose of EDC's effect on the metabolome



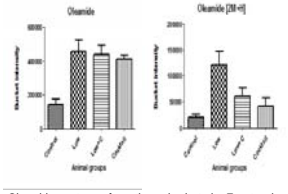
Un-targed metabolomics. The top 3 graphs shows unidentified metabolites from the methanol phase. The button three graphs are metabolites from the heptane phase. The main overall effect is an effect coursed by the cocktail.

Changes in phospholipid concentration



Changes in bucket intensity based on a anova test. As shown on the figure are the a lower level of phospholipids in the rats dose with both PFNA and cocktail than the other groups. This support the general idea than more EDC will effect the metabolome more than a single EDC.

Oleamide

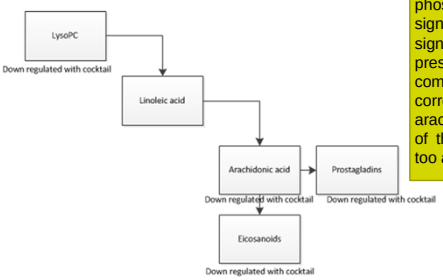


Oleamide contance from the animal study. Eventough, oleamide is a slippery agent it is interesting that there is a lower level of this compound in the control compared to the other 3 groups

Results

- The phospholipid phase shows some effect at the middle concentration. The main effect shown is a cocktail effect but there is also a trend of an additive effect.
- The analysis also reveals a compound verified by MS/MS to be oleamide. Oleamide is a slippery agent but also an endogenous compound and would therefore normally be discarded as an interesting compound. As the animals has been treated alike and as all blood taken from the animals it is interesting that there is a difference in oleamide between control and dosed animals.
- The un-target analysis shows primarily an effect from the cocktail. The main effect shown is a lowered amount of a given metabolite when the cocktail is given compared to control, though in some cases an overexpression is also shown.

Possible mechanism of cocktail



The analysis of the phospholipids show a significantly decrease in signal when cocktail is present. Furthermore, compounds with a mass corresponding to arachidonic acid and some of the prostagladins which too are down regulated.

Conclusion

- The phospholipid are significantly down-regulated when the rats are given PFNA and cocktail. Furthermore, is there a trend that the animals given both PFNA and cocktail have a larger down regulation that cocktail alone.
- The heptane phase shows a 'cocktail effect' – meaning that the animals are effected by a low dose cocktail. These compounds are believed to be mono- and di-glycerides but this is not yet varified by MS/MS
- The methanol also show a cocktail effect , though the metabolites are not yet identified.